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Research Article

Selective preconcentration of volatile mercaptans in small SPE cartridges: Quantitative determination of trace odor-active polyfunctional mercaptans in wine

A general procedure for the selective preconcentration and purification of mercaptans has been developed. Mercaptans are strongly retained in a small (20 mg) SPE cartridge containing *p*-hydroxymercurybenzoate. The cartridge can then be rinsed with relatively high volumes of polar (water/methanol mixtures) and non-polar (pentane or pentane/ether mixtures) rinsing solutions to remove nearly all volatile compounds lacking a thiol functionality. Retained analytes are further eluted with a small volume of an organic solvent containing 1,4-dithioerythritol. Some basic aspects of the strategy, such as the retention of *p*-hydroxymercurybenzoate in the sorbent and its stability *versus* different rinsing and eluting systems, have been studied in depth. Light sulfur compounds contained in water or wine, including mercaptans such as methanethiol or thioethers, such as diethyl sulfide, can be quantitatively extracted, although only mercaptans can be quantitatively recovered if a polar rinsing is applied. The strategy has been applied to the GC-MS quantitative determination of some trace polyfunctional mercaptans that are key aromas in wine, such as 2-methyl-3-furanthiol, 2-furfurylthiol, 4-mercapto-4-methyl-2-pentanone, 3-mercaptohexyl acetate or 3-mercaptohexanol. The developed method reaches detection limits in the ng/L range and has a satisfactory analytical behavior, being quite simple and fast.

Keywords: Aroma / Flavor / GC-MS / Mercaptans / SPE
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1 Introduction

Volatile sulfur compounds play an important role in various compartments of the environment and daily life [1] mainly because of their strong and unique odors. Some light sulfur compounds, particularly mercaptans, are the main cause of bad odors both in air and in water [2]. Furthermore, some of those light sulfur compounds are also related to odor or flavor properties, not only negative [3], of foodstuffs because of their characteristic smell and low sensory thresholds. On the other hand, the most powerful aroma molecules in nature are polyfunctional mercaptans, which are molecules combining a second chemical functionality in the molecule. Most of such polyfunctional mercaptans have very strong and distinctive smells and are responsible for the aroma and flavor of numerous vegetable species, such as box, blackcurrant, grapefruit [4], passion fruit [5, 6], onions [7, 8] and green tea [9], of some foods such as coffee [10, 11], meat [12–14], Iberian ham [15] and wine [16–19] and even have been described as majorly responsible for the odor of some species of mammals such as skunks [20], cats [21] or even humans [22]. There is an obvious need for analytical strategies not only for their quantitative determination, but also for their selective isolation.

The analysis of odor-active sulfur compounds is a challenging task not only because of the low concentrations at which these compounds can be perceived, but also because of their high reactivity and poor spectrometric properties. Because of this, many procedures for the quantitative determination of light mercaptans make use of derivatization strategies which are often considered more

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Abbreviations: DES, diethylsulfide; **DTE,** 1,4-dithioerythritol; **EtSH,** ethanethiol; **FFT,** 2-furfurylthiol; **HeptSH,** 1-heptanethiol; **HxSH,** 1-hexanethiol; **IS,** internal standards; **MeSH,** methanethiol; **MF,** 2-methyl-3-furanthiol; **MH,** 3-mercaptohexanol; **MHA,** 3-mercaptohexyl acetate; **MP,** 4-mercapto-4-methyl-2-pentanone; **PHMB,** *p*-hydroxymercurybenzoate; **PropSH,** propanethiol

robust and accurate [23–27] and only in few cases selective isolations of these analytes have been developed [28–30]. Nevertheless, in the case of polyfunctional mercaptans the opposite is true and most of the few proposed analytical procedures are based on the selective isolation of the underivatized mercaptans [11, 31–33] and only some recent reports make use of derivatization procedures [34–36] to determine these compounds as their pentafluorobenzyl derivatives.

The approaches for the selective isolation of underivatized mercaptans are compulsory for the unequivocal identification of the molecules responsible for aroma problems or aroma properties since the perception of the odor at a sniffing port operating in parallel to the mass spectrometer is crucial for avoiding misassignments. The most commonly used strategies for the selective preconcentration of mercaptans make use of the complexing properties of the thiol group to certain forms of organic mercury. One of the most common approaches, particularly in the flavor industry, is known as ''covalent chromatography''. This was proposed by Full and Schreier, and consists of percolating an extract through a small bed of agarose gel containing phenylmercuric ions [30, 37], which selectively binds mercaptans and is further eluted with a solvent containing a competitive mercaptan. A second strategy makes use of aqueous solutions of *p*-hydroxymercurybenzoate (PHMB) for the selective isolation of the mercaptans present in an extract. Complexed mercaptans are further purified using anion exchangers [11, 17]. Different versions of this idea have also been proposed in the recent literature [31, 33]. However, all these procedures are tedious, labor-intensive, difficult and slow and involve numerous phase-transferences of analytes, which not only makes them unattractive, but also seriously compromises their analytical performance due to the instability and reactivity of these compounds.

Any possible shortcut should limit the number and volume of phases to which mercaptans are transferred and in this sense it would be advantageous performing all the analytical operations (extraction, concentration and purification) in a single and small device. This article explores the ability of some common SPE sorbents to retain small amounts of organomercury salts (hazardous poison) for the development of fast, simple and highly selective quantitative preconcentration strategies of volatile mercaptans and presents an optimized quantitative procedure for the GC-MS determination of underivatized polyfunctional mercaptans potentially responsible for relevant sensory properties found in wines.

2 Materials and methods

2.1 Reagents and standards

n-Pentane for organic trace analysis (UniSolv), dichloromethane (SupraSolv), methanol (Suprasolv), ethanol, gradient grade for LC (LiChrosolv) and ortho-phosphoric acid 85% (Suprapur) were from Merck (Darmstadt, Germany). Anhydrous sodium sulfate, sodium chloride, L(+)-tartaric acid and acetic acid glacial for analysis, ACS-ISO quality, were from Panreac (Barcelona, Spain). EDTA, HEPES, 1,4-dithioerythritol (DTE), α,α,α-Tris, PEG 200, 4-(hydroxymercury)benzoic acid sodium salt (PHMB) were from Aldrich (Steinheim, Germany). 4-Mercapto-4-methyl-2-pentanone (MP) 1% PG, 4-methoxy-2-methylbutan-2-thiol, 3-mercapto-3-methyl-butylformate and 3-mercaptohexyl acetate (MHA) were from Oxford Chemicals (Hartlepool, UK). 2-Furfurylthiol (FFT), 3-mercaptohexanol (MH), 1-hexanethiol (HxSH) and 1-heptanethiol (HeptSH) were from Lancaster (Strasbourg, France). 2-Methyl-3-furanthiol (MF), 4-methoxy-α-toluenethiol, 2-phenylethanethiol, methyl benzoate, methyl phenylacetate and methyl hexanoate were from Fluka (Buchs, Switzerland). Methanethiol (MeSH), ethanethiol (EtSH), 1-propanethiol (1-PropSH), diethylsulfide (DES), ethyl methyl sulfide, isopropyl sulfide (2-PropSH), and ethanedial were supplied by Sigma-Aldrich (St. Louis, MO, USA).

To preserve integrity of sulfur standards, all solutions and samples were prepared, by weighing, in sealed vials protected from light, with solvents and vials purged with nitrogen, and always kept at low temperatures (−20°C solutions and 5°C for aqueous solutions). NaCl brine solutions were prepared dissolving 175 g of NaCl in 500 mL of water. Pure water was obtained from a Milli-Q purification system (Millipore, Billerica, MA, USA).

LiChrolut-EN sorbent was obtained from Merck and BondElut-ENV resins, prepacked in 50-mg cartridge (1 mL total volume) and the semi-automated SPE VAC ELUT 20 station were from Varian (Walnut Creek, CA, USA).

Standards and reagents for GFAAS (Graphite Furnace Atomic Absorption Spectrometry) method [38] were used to quantify the Hg eluted out of the cartridge. Hg and Pd solutions were prepared from commercially available 1 g/L single-standards (Merck), by appropriate dilution with 0.14 M HNO_3. $KMnO_4$ (3% w/v) solutions were prepared from the solid reagent (Ultrapure quality, Scharlau, Barcelona, Spain). In total, 14 M HNO_3 was purchased from Merck.

2.2 Methods

2.2.1 Retention properties of PHMB on polymeric sorbents

Aqueous loading solution: 2 mM PHMB in 0.1 M Tris, buffered at pH 7.2; some other loading solutions were also considered (2 mM PHMB buffered with HEPES 0.1 M at pH 7.2, with phosphate 0.1 M at pH 7.2 or with Tris 0.1 M at pH 10).

Aqueous polar washing-up solution: 5 g/L of tartaric acid, pH 3.0 and 40% of methanol; some other polar washing-up solutions were also considered (Tris 0.1 M at pH 7.2; acetate 0.033 M buffered at pH 4.5 always with 40% methanol).

Non-polar washing-up solutions: pentane and dichloromethane.

Loading solutions or washing-up polar or non-polar solutions were percolated through a cartridge (1 mL reservoir) containing 50 mg of BondElut-ENV sorbent from Varian or 20 mg of LiChrolut-EN sorbent from Merck at a maximum flow of 2 mL/min. In the case of the loading solutions, fractions of the eluate were taken (500 μL), diluted 40 times with Tris 0.1 M at pH 7.2 and analyzed to determine its total Hg content by GFAAS according to the method of Resano *et al.* [38]. The corresponding breakthrough curves representing the content of Hg in the fractions collected (mg/L) *versus* the volume of solution passed through the cartridge were built. When non-polar washing-up solutions were loaded, the eluate was directly analyzed in the GFAAS system.

Method for the determination of Hg by GFAAS. The empty platform was first transported to the microbalance, having the digits to 0.001 mg, using a pair of tweezers. After taring, the sample was placed onto the platform and weighed. The corresponding amount of chemical modifier (10 mL of 3% $KMnO_4$ solution) was added afterward. Finally, the platform containing the sample and the modifier was transferred into the graphite furnace and subsequently subjected to the temperature program. All these operations were fully controlled from a keyboard, except for the deposition of the sample and the modifier onto the platform, which was carried out manually. The operating conditions used are summarized in Table 1 of Resano's article [38]. Five replicate measurements were carried out for each determination and the median was taken as the representative value, in order to minimize the possible influence of outliers [38]. Calibration was carried out against aqueous standards solutions, added with a micropipette onto the sampling platform, together with the chemical modifier. A calibration curve (three standards and a blank) was measured in the beginning of every working session and, afterward, the measurements of all the solid sample replicates were carried out. In order to detect and correct for possible drifts in sensitivity, a standard solution was remeasured after ten solid sample replicates. Integrated absorbance was selected as the measurement mode in all cases.

Table 1. Amount of PHMB lost (expressed as percentage of the total loaded in the cartridge) after rinsing with different polar and non-polar washing-up and elution phases

Washing-up phase	Lost PHMB (%)
2.5 mL Tris pH 7.2	60
5 mL Tris pH 7.2	76
2.5 mL Acetate pH 4.5	20
5 mL Acetate pH 4.5	45
2.5 mL Tartaric acid pH 3	3
5 mL Tartaric acid pH 3	3
5 mL Pentane	0
5 mL DCM	0
5 mL 100 mM DTE in DCM	0

2.2.2 Retention properties of light sulfur compounds in beds containing PHMB

The breakthrough curves of different light sulfur compounds contained in water in the cartridges containing the Hg salt were built as follows: 200 mL of water spiked with 109 μg/L of MeSH (0.45 μmol), 190 μg/L EtSH (0.35 μmol), 76 μg/L 2-PropSH (0.29 μmol), 59 μg/L H×SH (0.18 μmol), 163 μg/L FFT (0.19 μmol) and 33 μg/L of DES (0.24 μmol) were percolated through a 20 mg LiChrolut-EN cartridge (1 mL reservoir) containing the Hg-salt retained by passing 1 mL of the aqueous loading solution through the cartridge. The eluate was sequentially collected into 25 mL fractions and the light sulfur compounds contained in each fraction were determined by HS-SPME-GC-PFPD as detailed in [39]. For this analysis, a 100 μL-volume of each fraction was introduced with a syringe into a sealed vial previously purged with N_2 and containing 100 mM cysteine in 4.9 mL of brine; 2-PropSH and isopropylsulfide were used as internal standards (IS) (10 μL of a 5.6 mg/L IS solution were added into the vial). Two different pHs of the loading aqueous solution were considered: pH 7.2 (Tris 0.1 M) and pH 3.0 (5 g/L of tartaric acid).

In order to build breakthrough curves for these compounds in different polar washing-up solutions, some experiments were carried out by loading the washing-up solutions containing the aforementioned light sulfur compounds at the concentrations cited before. Solutions considered were a 5 g/L (0.03 M) tartaric acid solution buffered at pH 3.0; a 0.03 M acetate solution buffered at pH 4.5 and a 0.03 M phosphate buffered at pH 6.0, containing all of them 40% methanol.

2.2.3 Procedure for the isolation of light mercaptans in water or wine samples

A volume of liquid sample (100 mL of water or 12 mL of wine) is loaded into a 20 mg LiChrolut-EN cartridge (1 mL reservoir) containing PHMB. After this, interferences are rinsed with 3 mL of a 40% methanolic solution at pH 3.0. The elution of mercaptans retained in the cartridge is carried out with 600 μL of 100 mM DTE in PEG200. Mercaptans are then analyzed by introducing 100 μL of this extract into the SPME vial and carrying the HS-SPME-GC-MS analysis as described in [39], but using an MS detector. The system used was a Varian CP-3800, Saturn 2200, ion trap MS, with a column DB-WAXetr from J&W of 60 m, 0.25 mm id and 0.25 μm of phase thickness.

2.2.4 Recommended procedure for the selective isolation of polyfunctional mercaptans in wine

(i) *Method optimization*. Different wine sample volumes (50–250 mL), washing-up polar solutions and volumes (aqueous solutions of Tris, tartaric, acetic or phosphoric acid at pH 7.2, 3.0, 4.5 or 6.0 respectively, and

containing 40% of methanol) and non-polar washing-up solvents and volumes (*n*-pentane, *n*-hexane and dichloromethane) were checked to ensure a quantitative extraction of the analytes and a large removal of interfering compounds from wine.

(ii) *Proposed procedure*. In total 20 mg LiChrolut-EN SPE cartridges were prepared in 1 mL standard polypropylene SPE reservoirs, with PTFE frits from Supelco; this preparation has been the same for all the experiments carried out. The sorbent was conditioned with 1 mL of dichloromethane, 1 mL of methanol, 1 mL of water and finally with 1 mL of a 0.1 M Tris solution, pH 7.2. After this, PHMB was fixed in the cartridge simply by slow percolation of 1 mL of 2 mM PHMB in Tris 0.1 M, pH 7.2 (aqueous loading solution). Then, 100 mL of wine (containing 5 g/L of EDTA and spiked with 200 ng/L of HeptSH, 500 ng/L 4-methoxy-α-toluene-thiol, 200 ng/L 4-methoxy-2-methylbutan-2-thiol and 200 ng/L 3-mercapto-3-methyl-butyl-formate as IS) were percolated through the cartridge at a maximum flow of 5 mL/min. After this, the cartridge was rinsed first with 1 mL of water, then with 5 mL of an aqueous solution containing 40% methanol (v/v) and 5 g/L of tartaric acid buffered at pH 3.0 and again with 1 mL of water. The sorbent was then dried by forcing a stream of pure N_2 (*ca*. 50 mL/min) to pass through the bed for 5 min. Non-polar impurities were further removed by rinsing with 6 mL of pentane. Complexes formed in the cartridge were eluted by adding 600 μL 100 mM DTE in dichloromethane with a glass syringe. The extract was washed with 5 mL of water and was then transferred to a 2 mL vial containing a small amount of anhydrous Na_2SO_4. In total 10 μL of the chromatographic IS solution (14.0 mg/L of methyl benzoate, 15.9 mg/L of methyl phenylacetate and 10.6 mg/L of methyl hexanoate) were added. The extract was concentrated by evaporation on a water bath (47°C) to a final volume of about 100 μL. In total 2 μL of this extract, injected in splitless mode, were analyzed by GC-MS in the conditions described below.

(iii) *GC-MS*. Apparatus: Shimadzu QP-2010 GC with a quadrupole MS detection system with a standard electron impact ion source. The column was a DB-WAXetr from J&W Scientific, 60 m × 0.25 mm id, with 0.25 μm film thickness. Initial oven temperature was 45°C for 2 min, heated to 110°C at 5°C/min, then to 230°C at 9°C/min and finally to 250°C at 15°C/min; remaining at that temperature for 15 min. The temperature of the ion source was 220°C and the interface was kept at 240°C. Data acquisition was by GC-MS using SIM at 0.40 s/point. From 11.0 to 13.0 min m/z 74 and 100 were acquired to quantify methyl hexanoate and 4-methoxy-2-methylbutan-2-thiol respectively; from 13.0 to 14.0 min, m/z 132 for HeptSH; from 14.0 to 16.8 min, m/z 114 and 81 for MF; from 16.8 to 18.0 min, m/z 132 and 75 to quantify MP; from 18.0 to 19.5 min, m/z 81 and 114 for FFT; from 19.5 to 21.0 min, m/z 102 for 3-mercapto-3-methyl-butylformate; from 21.0 to 23.0 min, m/z 105 was acquired to quantify methyl benzoate; from 23.0 to 27.0 min, methyl phenylacetate (m/z 91), MHA (m/z 116 and 82) and MH (m/z 100 and 88) were quantified; finally, from 27.0 to 31.0 min, m/z 121 was acquired to quantify 4-methoxy-α-toluenethiol.

(iv) *Validation of the procedure*. Method limits of detection and quantification were determined by the analysis of real samples (commercial wines poor in mercaptans), spiked at low concentration levels of analytes, as the concentration of analyte in wine which would give a signal three or ten times higher than the noise.

(v) *Calibration*. Four different wines (two white wines, a young red wine and an aged red wine) made from varieties poor in mercaptans (*macabeo* and *tempranillo*) were spiked with different masses of the analytes (four replicated levels plus the unspiked sample) and analyzed according to the proposed procedure. The peak areas obtained were normalized by those of the IS (3-mercapto-3-methyl-butylformate for MF and MH, 4-methoxy-2-methylbutan-2-thiol for MP and MHA, HeptSH for FFT). With the data, a calibration graph was calculated for each analyte. The slopes obtained in the analysis were averaged and used to estimate the concentrations of unknowns. Recovery experiments were carried out at two concentration levels in three different wines as summarized in Table 4.

3 Results and discussion

Previous works have suggested that PHMB and its complexes can be strongly retained in some polymeric sorbents typically used for reversed-phase SPE [31]. These kinds of sorbents can work both in aqueous and in organic-hydrophobic media retaining their chromatographic and retention properties and not suffering substantial changes when passing from aqueous to organic-hydrophobic solvent systems, a clear improvement when compared with hydrophilic resins, such as Affigel 501. Such properties could be exploited to develop specific methods in which the extraction, enrichment and purification could be carried out in a single and small cartridge. The general outline of such methods would be (i) fixing the Hg salt in the bed, (ii) loading the sample, (iii) solvent rinsing (polar and non-polar), (iv) elution and (v) GC-analysis. The key to the success of such a method is the understanding of the retention properties of PHMB in the SPE cartridge. These results will be first presented (Section 3.1) and two different applications will be further discussed in Sections 3.2 and 3.3.

3.1 Retention of PHMB in polymeric sorbents and stability versus rinsing/elution systems

PHMB has acid properties and can lose two protons in water solutions. At acid pHs, the neutral protonated form predominates and the molecule is not soluble in water. At neutral pHs, the molecule becomes a monovalent anion (p*K* for benzoate around 5.6) whose solubility depends on the nature of the buffer. Cationic buffers, such as Tris, enhance their solubility in comparison to anionic or inorganic buffers such as HEPES or phosphate. The strategy chosen for fixing PHMB in the sorbent was simply to extract it from a Tris solution buffered at pH 7.2. The breakthrough curves obtained for PHMB in two different sorbents are shown in Fig. 1 and, as can be seen, saturation of the LiChrolut-EN bed occurred at a volume above 4 mL. From this data it can be estimated that in these conditions a maximum of about 8 μmol (2.7 mg) of PHMB are retained in the 20 mg cartridge. In the case of BondElut-ENV resin saturation occurs at smaller volume in spite of the larger amount of sorbent (50 mg). The amount of PHMB that this resin can bind is, accordingly, smaller (about 6 μmol–2.1 mg) and because of this, LiChrolut-EN resins were used for the rest of the study. For most applications, a 20 mg cartridge containing the PHMB extracted from 1 mL of the aqueous loading solution was found to work satisfactorily. Figure 1 also confirms that PHMB will be easily removed out of the cartridge at alkaline pHs. This can be seen in the breakthrough curve obtained at pH 10, which shows that saturation is reached in the first fraction. This result can be attributed to the two negative charges of the salt at this pH (p*K* for the proton of phenol is around 9.0).

The following question addressed was the stability of the PHMB retained in the cartridge *versus* different polar and non-polar washing-up phases. The PHMB rinsed out of the cartridge (as percentage of the total) by different solvent systems is summarized in Table 1. The table shows that polar phases at neutral (pH 7.2) or not very acidic (pH 4.5) pHs are able to rinse the Hg salt out of the column, while at pH 3.0 there are no appreciable losses. Similarly, non-polar solvents, such as dichloromethane or pentane, even containing DTE, are not able to elute the PHMB contained in the sorbent. These results indicate that polar rinses at acidic pHs and non-polar rinses can be safely used to remove, respectively, polar or non-polar compounds retained in the cartridge not specifically bonded to Hg.

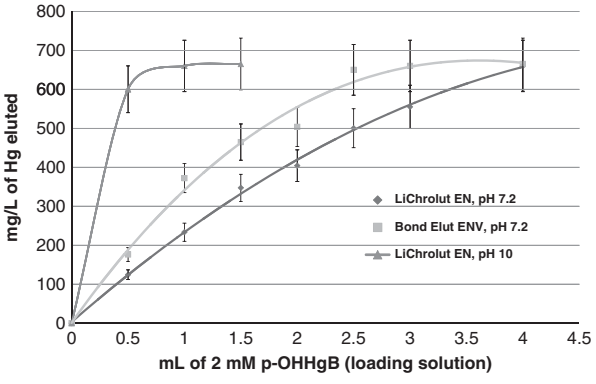

Figure 1. Breakthrough curves of PHMB in two micro-SPE cartridges (20 mg of LiChrolut-EN resins or 50 mg of BondElut-ENV resins) at two pHs of the aqueous (Tris 0.1 M) loading solution.

3.2 Selective preconcentration of light sulfur compounds

The ability of a 20 mg cartridge to retain different light sulfur compounds contained in water was checked by building the corresponding breakthrough curves as indicated in the procedures (see Section 2.2.2). Results at two different pHs are shown in Fig. 2, which shows an erratic behavior and a poor retention of nearly all compounds at neutral pHs. The erratic behavior can be attributed to the presence of the mercury salt in the effluent, which hinders the analysis of the compounds in the headspace. The poor retention is a direct consequence of the continuous losses of the mercury salt from the trapping system. The retention properties of the system are, however, very good at acidic pHs. As it can be observed, even the most hydrophylic compounds, such as MeSH and EtSH, can be completely retained and the breakthrough is not reached in more than 150 mL. Only diethylsulfide, that in fact is not a mercaptan but a thioether, is not well retained. Finally, at a volume around 200 mL, the system collapses because all the mercury trapped in the cartridge (2 μmol in this experiment) is already complexed and the system loses any ability to trap more mercaptans. All this suggests that retention is nearly infinite for all mercaptans contained in water, provided that the cartridge has enough mercury salt to trap all of them.

The possibility of using a rinsing solution in order to eliminate as many potentially interfering compounds as possible was also considered. For this study, the breakthrough curves for the analytes in polar washing-up solutions (40% methanol) at three different pHs were built and results are shown in Fig. 3. As it can be seen, MeSH and EtSH are not retained at pH 6.0, while mercaptans with more than five carbon atoms, such as FFT and HxSH, are still kept in the cartridge. This result is interesting, since it suggests that the complexes of PHMB with non-polar mercaptans are not eluted at neutral pHs. On the other hand, no loss of any mercaptan is noticeable at pH 3.0 and only diethylsulfide is eluted out of the system. At pH 4.5 the behavior is intermediate between those previously described and only small amounts of MeSH, EtSH and 1-PropSH are lost during the rinsing step. The strong dependence of retention with pH should be mainly attributed to the decreasing ability of the sorbent to retain the PHMB–mercaptan complexes at neutral or alkaline pHs, *i.e.* the complex is not broken by the rinsing solution, it is the complex which is slowly eliminated of the cartridge at those pHs.

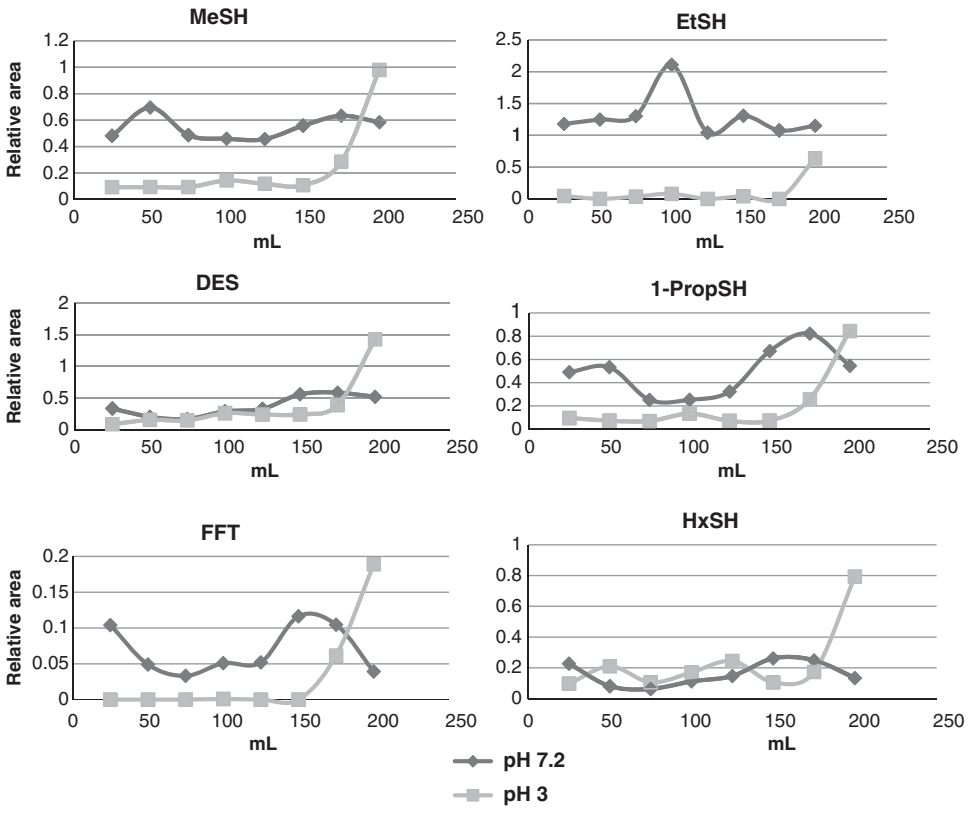

Figure 2. Breakthrough curves for different light sulfur compounds contained in water at pHs 3 and 7.2 in a 20 mg SPE cartridge containing PHMB.

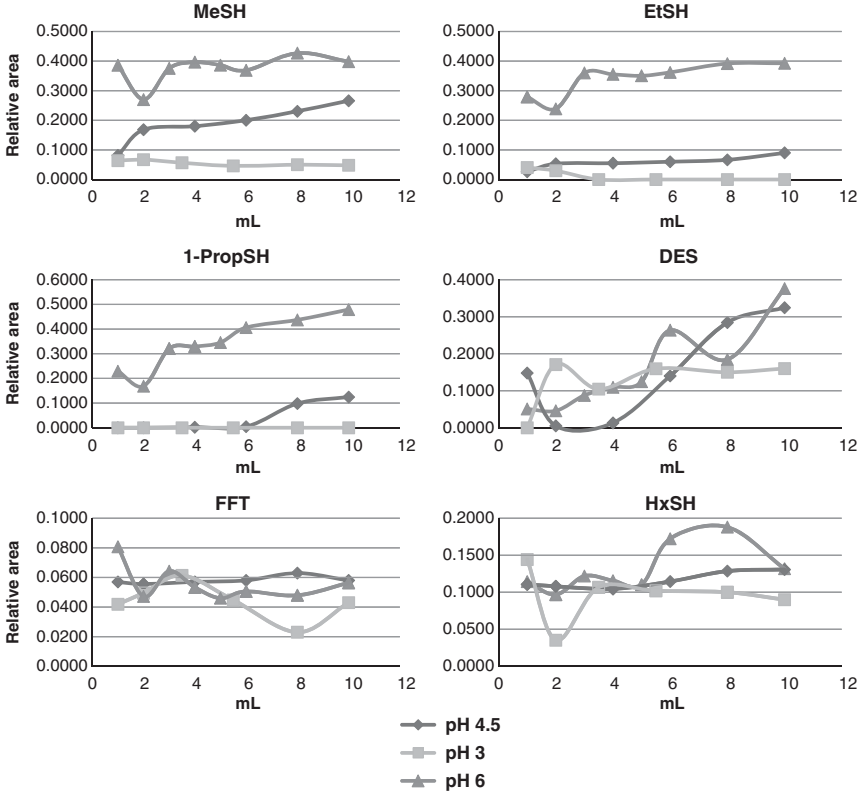

Figure 3. Breakthrough curves for the analytes contained in a polar rinsing solution at different pHs.

The elution of the mercaptans trapped in the microcartridge can be carried out under one of the following two different premises: (i) the elution of the entire Hg–mercaptan complex at alkaline pHs or (ii) the elution of the free mercaptan after cleaving the complex. This can be easily achieved by using a solvent, such as dichloromethane or PEG200, containing a relatively high level of DTE. For the analysis of light sulfur compounds, the use of a non-volatile water-miscible solvent such as PEG200 containing DTE (100 mM) is preferred to avoid coelution problems. A small volume of the elution solvent was directly diluted with brine and extracted by HS-SPME-GC-MS for the analysis of those light sulfur compounds (see Section 2.2.3).

The potential of the method is summarized in Table 2 which compares the GC-MS signals of some of the light sulfur compounds present in a wine, analyzed by direct HS-SPME-GC-MS of the wine with the results obtained applying the preconcentration step. It should be noted that only in this last case, clear and unequivocal mass spectra for the compounds could be obtained. As expected, preconcentration is very effective for mercaptans (MeSH, EtSH, 1-PropSH, HxSH and HeptSH) and less effective for thioethers (DES) and disulfides (DMDS). Results also show that in the case of mercaptans, recoveries are nearly quantitative for the system under study.

Table 2. Recovery of light sulfur compounds contained in 12 mL of a red wine preconcentrated according to the general procedure (Sections 2.2.2 and 2.2.3) including (ii) or not (iii) a washing-up step. For comparison, the signals obtained in the direct analysis (i) of the wine are also included in the same scale

Normalized signal	MeSH	EtSH	1-PropSH	DES	DMDS	HxSH	HeptSH
Direct (i)	2.0 ± 0.10	2.3 ± 0.11	2.5 ± 0.12	3.5 ± 0.17	1.0 ± 0.05	0	0
Preconcentrated and rinsed (ii)	70 ± 3.5	80 ± 4.0	79 ± 3.9	0.1 ± 0.01	7.8 ± 0.39	100 ± 5.0	97 ± 4.8
Preconcentrated (iii)	76 ± 3.8	73 ± 3.6	63 ± 3.1	19 ± 0.9	19 ± 0.95	98 ± 4.9	100 ± 5

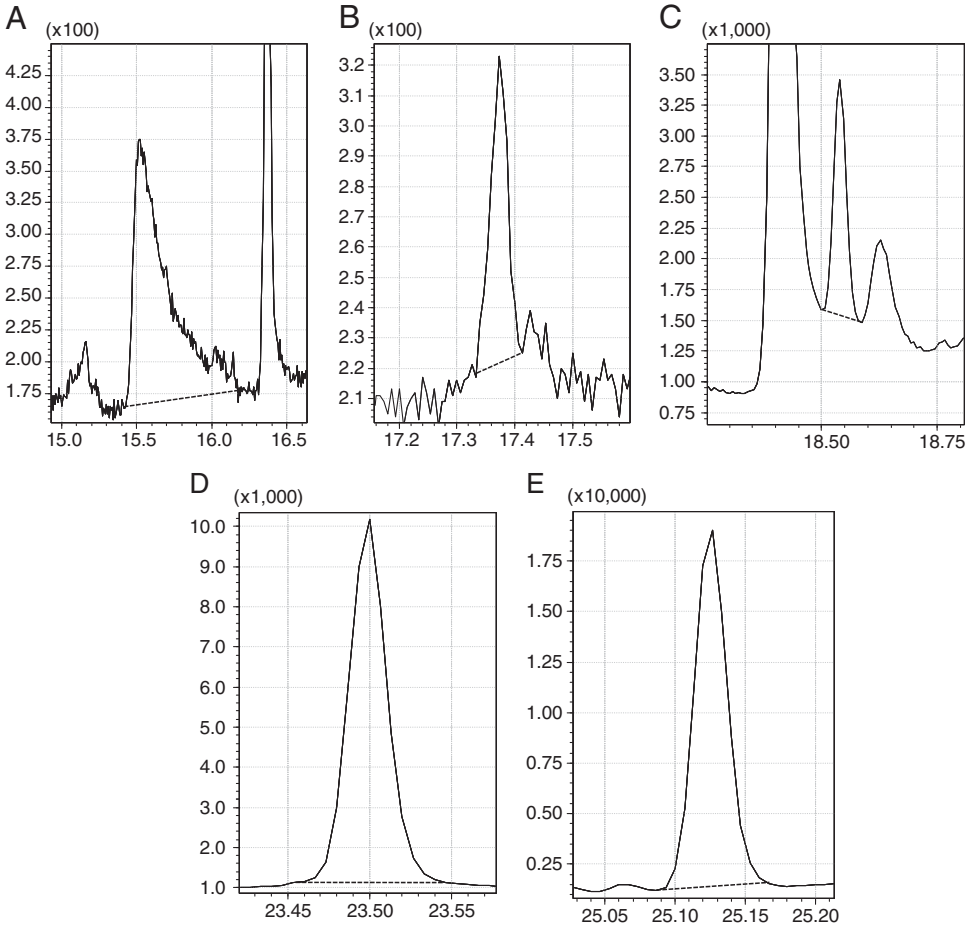

Figure 4. Ion chromatograms obtained in the analysis of white wine: A) MF, spiked at 100 ng/L, *m/z* 114; (B) MP, spiked at 15 ng/L, *m/z* 132; (C) FFT (unspiked), 2.5 ng/L, *m/z* 81; (D) MHA (unspiked), 25 ng/L, *m/z* 116; (E) MH (unspiked), 500 ng/L, *m/z* 100.

3.3 Development of a method for the selective isolation of polyfunctional mercaptans in wine

The usefulness of the approach has also been demonstrated by developing a quantitative procedure for the analysis at ng/L levels of some polyfunctional mercaptans playing outstanding roles in the sensory properties of many wines [31]. Preliminary experiments demonstrated that at the natural acidic pH of wines, the breakthrough volume of polyfunctional mercaptans was higher than 100 mL for the 20 mg SPE cartridge containing just 2 μmol of PHMB. The natural wine contents in cysteine and other mercaptans do not seem to be a limitation. The polar washing-up phase was optimized by considering the recoveries and rinsing effects achieved by different volumes of rinsing solutions with different pHs and percentages of methanol. Results showed that analytes retained in the cartridge are not lost after rinsing with polar solutions containing up to 70% of methanol, provided that the pH is kept low (data not shown). In practice, a 5-mL rinsing with a 40% methanol solution (approximately 250 bed volumes) buffered at pH 3.0 was found to be sufficient for the elimination of the major polar compounds and the obtained chromatograms were clear enough to quantify the target compounds.

Furthermore, different non-polar washing-up phases were also studied: *n*-pentane, pentane/diethylether, dichloromethane or dichloromethane/isopropanol. Best results were obtained with relatively large volumes of pentane (6 mL), since the use of the more polar phases brought about slight losses of analytes (data not shown). Elution was satisfactorily carried out with a small volume of dichloromethane containing DTE as displacing agent.

The final setup still had to face some practical problems related to the quality of blanks and to the apparition of distorted peaks in the chromatograms. The use of PTFE frits and the previous cleaning of reservoirs by immersion in dichloromethane for 24 h were found to be compulsory to get blanks allowing the detection of compounds at ng/L levels. Similarly, the cleanest chromatograms were obtained by treating the eluting extract with 5 mL of water to remove DTE.

The final procedure allows the determination of these key aroma compounds at very low levels in complex matrices with a quite simple sample work-up because sample manipulation is kept minimal. A typical chromatogram and some basic validation data about the application of the procedure for the quantitative determination of those compounds in wines are shown and summarized in Fig. 4 and Table 3, respectively. Detection limits are in the ng/L level, which can be considered acceptable according to the natural occurrence of these compounds in wine. The best detection limits are obtained for FFT and MP that are so far the analytes with lowest aroma detection thresholds. The repeatability was measured in a wine spiked with 400, 34, 8, 20 and 200 ng/L of MF, MP, FFT, MHA and MH, respectively, and the results obtained, summarized in Table 3, can be considered also acceptable. Worst results are obtained for MF and MH, both of which elute as tailing peaks under all tested

Table 3. Linearity and detection limits of the method for the quantitative determination of polyfunctional mercaptans in wine

Analyte	RSD %	LD (ng/L)	Average slope[a)]	Average[a)] R^2	Calibrated range (ng/L)
MF	9.0	12.0	$0.281 \times 10^{-3} \pm 7.0 \times 10^{-6}$	0.9901	25–500
FFT	6.5	1.5	$26.7 \times 10^{-3} \pm 6.4 \times 10^{-3}$	0.9989	2.5–50
MP	12.3	1.5	$0.87 \times 10^{-3} \pm 1.3 \times 10^{-4}$	0.9978	2.5–50
MHA	8.5	5.0	$5.37 \times 10^{-3} \pm 9.1 \times 10^{-4}$	0.9988	10–500
MH	7.8	13.0	$2.17 \times 10^{-3} \pm 4.5 \times 10^{-4}$	0.9967	25–1000

Results calculated with areas normalized to that of the corresponding internal standard for each analyte.
a) Average of four slopes (±mean standard error) calculated in four independent experiments in four different wines ($n = 5$).

Table 4. Method accuracy: recoveries (%) of polyfunctional mercaptans at two concentration levels in three different wines

Recovery (%)	Level (ng/L)	Sauternes	French red wine	Sauvignon Blanc
MF	10	111	118	47
	50	105	91	68
FFT	10	97	97	117
	50	89	98	110
MP	10	94	107	98
	50	97	110	108
MHA	10	90	112	74
	100	104	99	92
MH	30	28	123	78
	300	89	94	97

conditions. Method linearity is also satisfactory, except again in the case of MF ($0.990 < R^2 < 0.999$). Recoveries were assayed in three different wines at two concentration levels, and as can be seen in Table 4, were also satisfactory in most cases.

4 Concluding remarks

The possibility of fixing organo-mercury salts in polymeric sorbents makes it possible to expand the concept of ''covalent chromatography'' to standard SPE materials and procedures. In contrast to Affigel 501, a Hg-containing hydrophilic resin, the studied SPE sorbents, which have good chromatographic properties, can be directly exposed to large volumes of complex aqueous samples, and do not suffer strong structural changes when passing from aqueous to organic media. These properties greatly simplify selective preconcentration procedures for the qualitative or quantitative determination of mercaptans.

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